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(57) Abstract

The present invention relates to a vaccine comprising a *Lactobacillus* species that contains a nucleotide sequence that encodes a urease peptide capable of initiating an anti–urease humoral and/or cellular immune response upon administration to a mammalian species.

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ORAL VACCINE

The present invention relates to modified Lactobacilli and their use in the treatment of gastric disorders.

Gastric disorders such as gastritis, peptic ulcer disease etc. have recently been discovered to be due to the presence of Helicobacter strains in the stomach, particularly Helicobacter pylori. Consequently, much effort has been invested in the research for agents that will minimise the effect of Helicobacter strains and specifically H. pylori.

The results of this research include many chemical and biological agents that are designed to inhibit the bacteria, its activity and colonisation of the stomach. Although H pylori has been shown to be susceptible to many agents in in vitro tests, in vivo eradication has not often been achieved (Czinn SJ et al., Infect. Immunol. (1991) 59 2359-2363).

Urease is the most prominent protein component of Helicobacter pylori and it has been proposed that urease is produced in order to hydrolyse urea thus increasing the pH of the environment to one favourable to colonisation by Helicobacter (Mobley HLT et al., Microbiol Rev (1995) 59 451-480).

Thus, attempts have been made to eliminate or control H pylori by the administration of urease through various means. Pallen MJ & Clayton CL (The Lancet (1990) 336 186-7) suggested oral immunisation using plant urease, an idea carried out by Chen M et al. (FEMS Microbiol Lett. (1994) 116 245-250) using jack bean urease and a cholera toxin adjuvant. This and many other related strategies are described in Mobley HLT et al. supra.

International Patent Applications Publication Nos. WO95/22987 and WO96/33732 both describe urease based vaccines that utilise recombinant urease.

In both cases the cloning of the urease gene or fragments thereof, has been utilised as a method of producing sufficient urease to be administered together with a pharmaceutical carrier. Neither application discloses the oral administration of the expression vector itself for expression to occur in vivo.

The hope offered in utilising urease as a vaccine has not yet been fully realised due to difficulties in designing an efficient system for delivering sufficient urease to the desired site to stimulate a humoral and/or cellular immune response, in particular to initiate the production of secretory IgA, without undesirable or unwanted side effects to the recipient, due for example to an adjuvant.

The present invention therefore aims to provide a vaccine that is capable of delivering sufficient urease to the desired site such as to control/eradicate and/or prevent Helicobacter colonisation and thus treat Helicobacter related diseases.

Accordingly, the present invention relates to a vaccine comprising a Lactobacillus species that contains a nucleotide sequence that encodes a urease peptide capable of initiating an anti-urease humoral and/or cellular immune response upon administration to a mammalian species, preferably initiating the production of secretory IgA.

Suitable Lactobacillus species include any species or subspecies such as Lactobacillus delbrueckii and subspecies bulgaricus and lactis, Lactobacillus GG, a strain of L casei subspecies rhamnosus (Goldin et al., (1992) Dig Dis and Sciences 37 121-128), L fermentum, L. planatarum, L paraplantarum, L pentosus, L coryniformis, L casei, L paracasei, L brevis, L leichmannii and strains of Lactobacillus isolated from intestinal flora such as Lactobacillus rhamnosus 901. Lactobacillus rhamnosus 901 is of particular benefit as it possesses resistance to both acid and bile juices. In accordance with the present invention, the Lactobacillus spp is been modified such that the organism contains a nucleotide sequence either on a plasmid or in a chromosome capable of expressing a urease peptide as defined above.

Preferably, the Lactobacillus strain used is L. planatarum. More preferably, the strain of L. planatarum is selected from L. planatarum NCIMB 8826, NC4Ts1, NC7Ts5 or ATCC 8014, most preferably the NCIMB strain 8826 of L. planatarum is used.

Urease is a protein that is encoded by an array of genes that are comprised of structural, accessory and regulatory subunits. These are all required in order to produce a biologically active enzyme, for example certain subunits are involved with the incorporation of nickel into the protein structure that is essential for enzymatic activity. As discussed below, the retention of enzymatic activity is not essential for the purposes of the present invention.

The nucleotide sequences of use in the present invention must encode at least a fragment of urease capable of initiating an anti-urease humoral and/or cellular immune response upon administration to a mammalian species. Such an immune response is defined herein as the ability to initiate the production of anti-urease antibodies, in particular systemic and local IgA, detectable by methods known in the art, preferably by ELISA.

Suitable nucleotide sequences that are able to express a urease peptide capable of initiating a anti-urease humoral and/or cellular immune response include those that encode the whole urease protein, fragments thereof, homologs or analogs thereof. The full nucleotide sequences of H. pylori urease, including the genes encoding the structural subunits A and B (ureA and ureB), were reported in Clayton CL et al. Nucleic Acid Research (1990) 18 362 and these sequences may be used together or individually. It should be noted that the Helicobacter spp ureA peptide represents a fusion of ureA and ureB peptides in other microorganisms whereas the Helicobacter spp UreB peptide and H pylori UreH peptide are homologous to UreC and UreD peptides in other bacteria. Mobley HLT et al., Microbiol Revs, (1995), vol 59, pages 451-480, provides a detailed description of known urease sequences and any of these sequences in whole or

part that is capable of initiating an anti-urease immune response as defined above, may be used in the present invention. A non-exhaustive list of nucleotide sequences encoding urease is given in Table 1. As used hereinafter, all reference to urease subunits relate to the nomenclature used for H. pylori in Clayton CL et al. (supra).

Table 1

Species	Genes Sequenced	GenBank Accession No.	References
Complete gene cluster sequenced			
Bacillus sp. strain TB-90 H.	*ure*ABCEFGDHI	D14439	Maeda M, *et al* 1994, J. Bacteriol, 176:432-442
H. pylori[c]	*ure*,ABIEFGH	M84338, X57132, X17079, M60398	Clayton C, *et al* 1990, Nucleic Acids Res. 18:362, Cussac V, *et al* 1992, J. Bacteriol, 174: 2466-2473., Labigne A, *et al* 1991, J. Bacteriol, 173:1920-1931.
K. aerogenes	*ure*DABCEFG	M55391, M36068	Lee M.H, *et al* 1992, J. Bacteriol, 174:4324-4330. Mulrooney S.B. *et al* 1990, J. Bacteriol, 172:5837-5843.
P. mirabilis	*ure*RDABCEFG	M31834, Z18752, Z21940	Jones B.D, *et al* 1989, J. Bacteriol, 171:6414-6422. Nicholson E.B, *et al*1993, J. Bacteriol, 175:465-473. Sriwanthana B, *et al* 1993, G. Gene, 129:103-106.
Y. enterocolitica	*ure*ABCEFGD	Z18865, L24101	de Koning-Ward T.F *et al* 1994, Gene 145:25-32 Skurnik, M *et al* 1993, Immun. 61:2498-2504.
Complete Sequence for Selected Genes			
B. pasteurii	ure*ABC*	X78411	Morsdorf G, *et al* 1994, *Bacillus pasteurii.* GenBank accession No. x78411
H. felis	*ure*AB	X69080	Ferrero, R.L. *et al* 1993, Mo. Microbiol, 9:323-333.
H. heilmannii	*ure*AB	L25079	Solnick J.V *et al* 1994, Infect. Immun, 62:1631-1638
K. pneumoniae	*ure*DA	L07039	Collins C.M. *et al*1993, Mol.Microbiol. 8:187-198
L. fermentum	*ure*ABC	D10605	Suzuki K. *et al* 1992. *Lactobacillus fermentum* JCM5869. GenBank accession No. D10605
P. vulgaris	*ure*ABC	X51816	Morsdorf G *et al* 1990, FEMS Microbiol, Lett: 66:67-74
R. meliloti	*ure*DABC	S69145	Miksch G. 1994, FEMS Microbiol, Lett: 124:185-190 Miksch G, *et al* 1994, Mol. Gen. Genet. 242:539-550
S. xylosus	*ure*ABC	X74600	Jose, J, *et al* 1994, Arch. Microbiol, 161:384-392
U. urealyticum	*ure*ABC	X51315	Blanchard, A. 1990 Mol. Microbiol 4:669-678

The nucleotide sequences of use in the present invention, may be used alone or as part of a larger sequence encoding a fusion protein comprising a urease moiety or peptides homologous with urease. For example, urease B subunit may be expressed as a chimeric protein together with the cholera toxin B subunit by the insertion into a Lactobacillus spp of the urease B subunit nucleotide sequence linked to the cholera toxin B subunit nucleotide sequence.

As discussed above, nucleotide sequences that are homologous to a known urease encoding sequence may be used in the present invention. Preferably, such a sequence bears at least 70% homology to the H. pylori nucleotide sequence, more preferably at least 80% and most preferably at least 90 or 95% homology. For example, urease such as that encoding for jack bean urease bears about 70% homology with H pylori urease.

It is preferable to use a nucleotide sequence derived from H. pylori, but alternative sources of suitable nucleotide sequences include other Helicobacter species such as H felis, H heilmannii or Morganella morganii. Preferably, the nucleotide sequence encodes for at least the urease A or B subunits or A and B subunits. More preferably the nucleotide sequence encodes for at least the urease B subunit.

The urease peptide may possess urease activity or alternatively be devoid of such activity.

The nucleotide sequence may be inserted into the Lactobacillus genome or Lactobacillus plasmid as a single entity or in clusters linked together such as to produce a multimeric protein comprising for example, from two to eight urease A subunits and from two to eight urease B subunits. Within this embodiment, when more than one subunit is inserted into Lactobacillus, they may be inserted in equal or unequal numbers.

In preparation of a vaccine of the present invention, the appropriate nucleotide sequence capable of expressing a urease peptide may be amplified and isolated from a suitable source using PCR, fused with a Lactobacillus or Lactococcus promoter, ligated into a vector, plasmid or transposon such as Tn916 and then introduced into the Lactobacillus strain by for example, electroporation. An example of a suitable method for inserting a foreign gene into a Lactobacillus genome is given in European Patent Application 603416A, wherein a DNA cassette is formed comprising Lactobacillus DNA fragments from upstream and down stream of the proposed chromosomal integration site that are ligated to either end of the nucleotide sequence to be inserted, and the cassette inserted into pAMβ1 plasmid which is used as an integration plasmid. This plasmid is then introduced into a Lactobacillus spp such as L delbueckii by conjugal transfer to obtain transconjugants by integration into chromosomal DNA, that exhibit erythromycin resistance (originating from the plasmid). The subculturing is then repeated to generate subclones that become sensitive to erythromycin as a result of losing the relevant sequnce from pAMβ1 and from these subclones selecting those that contain the inserted nucleotide sequence.

Further methods of inserting nucleotide sequences into L plantarum include those disclosed in Hols P et al., (Microbiol (1997) 143, 2733-2741) that describes the insertion of sequences encoding the N-terminal portion of the Streptococcus pyogenes M6 portein fused to an epitope of the HIV gp41 protein into L plantarum NCIMB 8826, and Cosby WM et al., (Plasmid (1989) 22, 236-243) that describes the electroporation of the pTV1Ts temperature sensitive plasmid carrying the macrolide-lincosamide-steptogramin B resistance transposon Tn917 into L plantarum.

The transformed Lactobacillus may be used directly in the vaccine composition. Thus, an advantage of the present invention is that the vaccine is easily prepared not requiring the lengthy purification involved when handling recombinant proteins. The vaccine may be given alone or preferably included in foodstuffs that already contain a Lactobacillus spp such as yoghurt, fermented milk drink or

cheese etc. as the sole source of Lactobacillus or in addition to the existing strains.

The vaccines of the present invention may be used in the treatment of gastrointestinal (duodenal) disorders including gastritis, peptic ulcer disease including both gastric and duodenal ulcers, gastric cancer, chronic dyspepsia with severe erosive gastroduodenitis, refractory ulcer dyspepsia, intestinal metaplasia, low grade MALT lymphoma, Helicobacter infection, Helicobacter pylori infection and Helicobacter felis infection.

The vaccines may further contain pharmaceutically acceptable excipients such as adjuvants, solvents, preservatives, stabilisers and the like. Furthermore, the vaccine may additionally contain other pharmacologically active ingredients such as antibiotics, antisecretory agents and bismuth salts.

A further aspect of the present invention relates to transformed Lactobacillus spp. containing a nucleotide sequence that encodes a urease peptide as defined above capable of initiating an anti-urease cellular and/or humoral immune response upon administration to a mammalian species.

In use, the vaccine may be administered by methods known in the art. Thus it may be given by intravenous (I.V.), intramuscular (I.M.), subcutaneous (S.C.), intradermal (I.D.) or oral routes and the like. The dose is administered at least once. Subsequent doses may be administered as indicated. It is a particular advantage of the present invention that the vaccine may be administered orally. This advantage is assisted by the incorporation of Lactobacillus spp being incorporated into foodstuffs as discussed above. Administration of the vaccine may involve a priming dose, optionally by an alternative route to the main

dosage. For example, when the vaccine is to be adminstered orally, a subcutaneous priming dosage may be administered.

In providing a mammal, preferably a human, with the vaccine of the present invention, the dosage will vary depending upon such factors as the mammal's age, weight, height, sex, general medical condition, previous medical history, disease progression and the like.

EXAMPLES

Example 1; Transformation of L. plantarum

L. plantarum NCIMB strain 8826 was readily transformed by electroporation using a variety of plasmids including pNZ12, pNZ17, pNZ10α1, pTG3237 (all obtained from Netherlands Institute for Dairy Research (NIZO), Bostbus 20, 6710 BA, Ede, Netherlands). The highest transformation rate was achieved with pNZ10α5 where transformants typically arose at a frequency of $>10^3$ per ug of plasmid DNA.

Optimum recovery of plasmid DNA was achieved with Lactobacillus plantarum strain NCIMB 8826 and the vector pNZ10α5. 13μg DNA/100 ml culture is recovered using a modification of the method suggested by Frere (Letters in Applied Microbiology 18 227-9). In this modification, cell suspensions are incubated at 37°C for one hour and then vortexed for 1 min with an equal volume of glass beads (0.1-0.15 mm diameter) to disrupt the cell wall.

Once electroporated into strain 8826, pNZ10α5 has been found to be stable as shown by repeated subculture for up to 86 generations.

Example 2; Colonisation studies of Lactobacillus spp in BALB/c mice. Results and approach using Lactobacillus plantarum strain NCIMB 8826 and the vector pNZ17

9 BALB/c mice are divided into 3 groups and fed with pNZ17-transformed Lactobacilli. Transformants of Lactobacillus GG, Lactobacillus Sp. 901, and L. planatarum 8826 containing pNZ17 are prepared as described in Example 1, and then cultured in skimmed milk.

A single colony of each species of Lactobacilli is transferred into 100 ml of MRS (de Man, Rogosa & Sharpe) broth containing chloramphenicol 20μg/ml, respectively. The cultures are incubated overnight at 37°C without shaking.

Bacterial suspensions are harvested by centrifugation at 5,000g for 15 min at room temperature, the supernatant decanted and the bacterial pellet resuspended in 20 ml of skimmed milk.

Viable counts of each strain are prepared by pipetting 100µl of each bacterial suspension in 900µl MRS broth and then making serial 10-fold dilution. 100µl of each dilution is plated onto MRS agar plates and the number of colonies counted after 48 hours of incubation at 37°C.

Typical concentrations of Lactobacilli in skimmed mik were:
Lactobacillus GG: 3×10^8 cfu/ml - 2×10^9 cfu/ml,
Lactobacillus 901: 2×10^9 cfu/ml,
Lactobacillus plantarum NCIMB 8826: 3×10^9 cfu/ml.

Mice are fed 1 ml of lactobacilli-milk twice daily for 18 days and faeces collected three times a week for culture on MRS agar plates with and without chloramphenicol 20 µg/ml.

During feeding, Lactobacilli is detected in the faeces of all mice at high concentrations

Lactobacillus GG	2×10^7 cfu/g
Lactobacillus Sp. 901	1.2×10^7 - 1×10^8 cfu/g
Lactobacillus Plantarum 8826	7.4×10^7 - 1.2×10^8 cfu/g

24 h after cessation of feeding:

Lactobacillus GG	not detected
Lactobacillus Sp. 901	not detected
Lactobacillus plantarum 8826	2-3 x 10^8 cfu/g

5th day after cessati n of feeding

Lactobacillus plantarum 8826 still detectable at a concentration of 7 x 10^2 cfu/g

Culture of tissue from killed BALB/c mice,

24h after cessation of feeding

Lactobacillus GG

5 x 10^2 cfu/g small intestine

4 x 10^3 cfu/g caecum

3.5x10^3 cfu/g colon

Lactobacillus 901

1.35x10^3 cfu/g caecum

2.6 x 10^5 cfu/g colon

Lactobacillus plantarum 8826

5 x 10^3 cfu/g small intestine

8.6 x 10^7cfu/g caecum

2.5x10^6 cfu/g colon

Example 3; Transformant plasmids

Urease:

DNA encoding the structural subunits of urease gene, ureA and ureB (Clayton CL supra) is amplified by use of the polymerase chain reaction using Pfu *Taq* polymerase to ensure error free amplification. The primers used are;

YR1 : 5' AAGGAT TTAAGGAGCGTTGC 3 and,

YR2 : 5' GATTTCGTTATGTCTTCAAGG 3'

Alternatively, plasmid pTCP3 containing a 2.6kb insert encoding both the 66kDa ureB and 31kDa ureA subunits is used (Clayton CL et al and Infect Immun (1989) 57(2), 623-629). If so, pTCP3 is prepared by transformation into E. coli JM109 and digested using Taq1 to release the urease encoding fragment.

Construction of the secretion plasmid in pNZ10α5

pNZ10α5 carries the amyS gene fused to part of the *L. lactis* promoter gene usp45 gene (van Asseldonk et al 1993 Molecular and General Genetics 240, 428-434).

A secretion plasmid containing translational fusions between usp45 from Lactococcus lactis and the ureA+B or ureB gene is constructed. This encodes a highly secreted protein which maximises exposure to the host's mucosal immune system.

Fusion expression is driven by the promoter (-35 and -10) sequences of usp45, its ribosome binding site, translational start site and signal peptide (27 aa from ATG to Ala 27 which is the cleavage site).

To construct the urease secretion plasmid, pNZ10α5 is cut with PstI and HindIII to provide the first 57bp (19 first aa) of the signal sequence. A synthetic linker containing the final 24bp (8 aa) of the signal sequence and a restriction site is inserted into which the ureA and ureB or ureB only gene derived from PCR products or pTCP3 are cloned.

Successful insertion and expression of sequences encoding urease subunits is confirmed by Western blotting as described below.

Example 4; Detection of anti-urease antibodies

Anti-urease antibodies is detected in the sera of H pylori infected animals by ELISA as described in Tanaka K et al., Gut (1991) 32, 43-45.

Microtest III plates (Becton-Dickinson) are coated with urease at a concentration of 0.04 mg/ml in carbonate/bicarbonate buffer pH 9.6. The plates are incubated overnight at 4°C and then washed three times with phosphate buffered saline (PBS) pH 7.4 containing 0.05% (v/v) Tween20 (PBS/T20). Bovine serum albumin (1%w/v) (Sigma) in PBS pH 7.4 (PBS/BSA) is added to the wells to reduce non-specific binding. Plates are incubated for two hours at room temperature, washed three times with PBS/T20, and stored at -20°C until needed. 100µl of mouse serum is diluted 1:50 with PBS/BSA and added to the wells. After incubation overnight at 4°C, the plates were washed three times with PBS/T20. 100µl of anti-mouse IgG conjugate diluted according to the manufacturer's instruction is added to the wells. The plates are incubated for two hours at room temperature and washed three times with PBS/T20. A total of 100µl of 1 mg/ml alkaline phosphate substrate (Sigma 104) in substrate buffer pH 8.9 consisting of 0.2 M $NaCO_3$, 0.2M $NaHCO_3$, 0.01M $MgCl_2$ is added and incubated for one hour at room temperature. The reaction is stopped with the addition of 100µl of 1N NaOH and the colour read spectrophotometrically at 405nm using a Dynex MR1 ELISA reader.

Highly pure urease for coating the wells may be obtained from H. pylori using the methods described in Icatlo FC et al., J Biol Chem (1998) 273(29), 18130-18138.

Western blotting, is performed as in Tanaka K et al., supra with the modification that whole cell preparation of the Helicobacter pylori NCTC 11637 or 630 is used. The protein concentration is determined by a modified Lowry technique, protein dissolved in 10% sodium dodecyl sulphate and approximately 25µg of total protein is loaded in each lane and electrophoresed in a Hoefer Transblotter in a discontinuous buffer system at a constant current of 15 mA. Mice sera from vaccinated animals is diluted and tested at dilutions of 1/25 and 1/50.

CLAIMS

1. A vaccine comprising a Lactobacillus species containing a nucleotide sequence that encodes a urease peptide capable of initiating an anti-urease cellular and/or humoral immune response upon administration to a mammalian species.

2. A vaccine according to claim 1, wherein the nucleotide sequence encodes the whole urease protein, fragments, homologs or analogs thereof.

3. A vaccine according to claim 1 or 2, wherein the nucleotide sequence encodes structural subunits of urease.

4. A vaccine according to any preceding claim, wherein the nucleotide sequence is derived from a Helicobacter species.

5. A vaccine according to claim 4, wherein the Helicobacter specie is Helicobacter pylori.

6. A vaccine according to any preceding claim, wherein the nucleotide sequence encodes for at least the urease A or B subunits or A and B subunits.

7. A vaccine according to claim 6, wherein the nucleotide sequence encodes for at least the urease B subunit.

8. A vaccine according to any preceding claim, wherein the nucleotide sequence encodes a fusion protein.

9. A vaccine according to claim 8, wherein the fusion protein contains a cholera toxin B subunit.

10. A vaccine according to any preceding claim, wherein the Lactobacillus spp is selected from Lactobacillus delbrueckii, subspecies bulgaricus and lactis, L

fermentum, L. planatarum, L paraplantarum, L pentosus, L coryniformis, L casei, L paracasei, L brevis, L lechmannii Lactobacillus GG, a strain of L casi subspecies rhamnosus, Lactobacillus rhamnosus 901 and strains of Lactobacillus isolated from intestinal flora.

11. A vaccine according to claim 10, wherein the Lactobacillus spp is Lactobacillus plantarum.

12. A vaccine according to claim 11, wherein the Lactobacillus plantarum is NCIMB strain 8826.

13. A vaccine according to any preceding claim capable of initiating a cellular and humoral anti-urease immune response.

14. A vaccine according to any preceding claim capable of initiating production of secretory IgA.

15. Use of a vaccine as defined in any of claims 1 to 14 in the treatment of a gastrointestinal disorder.

16. Use according to claim 15, wherein the disorder is gastritis, peptic ulcer disease including both gastric and duodenal ulcers, gastric cancer, chronic dyspepsia with severe erosive gastroduodenitis, refractory ulcer dyspepsia, intestinal metaplasia, low grade MALT lymphoma, Helicobacter infection, Helicobacter pylori infection and Helicobacter felis infection.

17. A foodstuff containing a vaccine as defined in any of claims 1 to 14.

18. A foodsuff according to claim 17, in the form of a milk product.

19. A foodsuff according to claim 18, in the form of a yoghurt.

20. A transformed Lactobacillus spp. containing a nucleotide sequence that encodes a urease peptide capable of initiating an anti-urease cellular and/or humoral immune response upon administration to a mammalian species.

21. A transformed Lactobacillus according to claim 21, that is Lactobacillus plantarum.

22. A transformed Lactobacillus according to claim 20 or 21, wherein the nucleotide sequence encodes at least the ureB subunit.

23. A vaccine according to any of claims 1 to 14 capable of oral delivery.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 654 273 A (LEVEEN H. ET AL.) 24 May 1995 see column 4, line 8 - column 4, line 43 see column 11, line 27 - column 12, line 40 see column 13, line 16 - column 13, line 32 see claims 28,34-36 --- -/--	1-3,10, 11, 13-16,23

[X] Further documents are listed in the continuation of box C.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 December 1998	22. 01. 99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Luzzatto, E

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

In Application No
PCT/GB 98/02631

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	POUWELS P.H. ET AL.: "The potential of Lactobacillus as a carrier for oral immunisation: development and preliminary characterisation of vector systems for targeted delivery of antigens" JOURN. BIOTECHNOL., vol. 44, 1996, pages 183-192, XP000572655 AMSTERDAM, nl see page 184, left-hand column, line 18 - left-hand column, line 41 see page 185, left-hand column, line 1 - left-hand column, line 30 see page 190, right-hand column, line 17 - page 191, left-hand column, line 26 ---	1-23
Y	WO 96 33732 A (ORAVAX,INC.) 31 October 1996 cited in the application see page 2, line 1 - page 6, line 21 see page 25, line 1 - line 14 see claims ---	1-23
A	WO 94 16086 A (BIOTEKNOLOGISK INSTITUT) 21 July 1994 see page 5, line 5 - page 8, line 24 see page 27, line 30 - line 33; claims -----	1-23

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 98/02631

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 15,16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int Application No
PCT/GB 98/02631

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 654273 A	24-05-1995	NONE	
WO 9633732 A	31-10-1996	AU 5576496 A	18-11-1996
		CA 2219201 A	31-10-1996
		CZ 9703426 A	17-06-1998
		EP 0831892 A	01-04-1998
		HU 9801266 A	28-08-1998
		NO 974969 A	23-12-1997
		PL 323048 A	02-03-1998
		US 5837240 A	17-11-1998
WO 9416086 A	21-07-1994	AU 675821 B	20-02-1997
		AU 5832594 A	15-08-1994
		CA 2152898 A	21-07-1994
		EP 0677110 A	18-10-1995
		JP 8500739 T	30-01-1996
		NZ 259510 A	24-06-1997
		NZ 286635 A	24-06-1997
		US 5837509 A	17-11-1998